

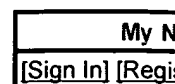
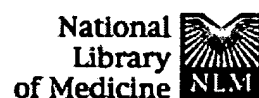
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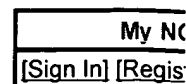
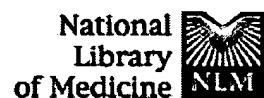
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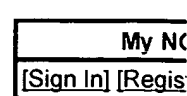
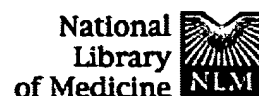
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Antigenic expression of human melanoma cells in serum-free medium.

Bumol TF, Harper JR, Chee DO, Reisfeld RA.

A human melanoma cell line, M14, adapted to grow in serum free synthetic media was examined for its expression and secretion of several serologically defined melanoma associated antigens (MAA) previously described in this laboratory. Melanoma associated antigen expression and secretion was identical to that of M14 cells grown in parallel in serum supplemented medium. Spent synthetic media was found to be an enriched serum free source for the initial isolation of 100 kilodalton secreted glycoprotein MAA. M14 melanoma cells grown in synthetic media were also shown to be adaptable to the double agar clonogenic assay facilitating the examination of clonal heterogeneity in functional studies of MAA in melanoma tumor biology. Recent investigations from this laboratory have focused on characterizing human melanoma associated antigens (MAA) found either as secreted or cell surface associated glycoproteins in human melanoma cell lines. In these studies, monoclonal and polyclonal antisera to melanoma cell components have been developed to specifically identify these MAAs immunochemically and provide a means to study the structural biochemistry of these determinants. At this time we have identified two antigens on which our research efforts are targeted: 1) a 100,000 dalton secreted glycoprotein (100K) common to melanoma, sarcoma and neuroblastoma tumor cell lines, and 2) a 250,000 dalton-high molecular weight component glycoprotein-proteoglycan complex which is thus far restricted to melanoma cells. The ultimate goal of our efforts is two-fold. Initially, we hope to develop schemes to isolate these melanoma associated antigens in sufficient quantities to obtain detailed structural information on these molecules, and secondly, we wish to implicate these glycoproteins in functional aspects of the biology of metastatic human melanoma in vitro. (ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 6731148 [PubMed - indexed for MEDLINE]



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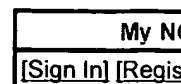
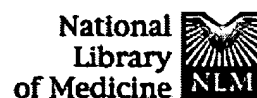
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Studies of a melanoma tumor-associated antigen detected in the spent culture medium of a human melanoma cell line by allogeneic antibody. I. Purification and development of a radioimmunoassay.

Gupta RK, Morton DL.

A tumor-associated antigen (TAA) was isolated from spent culture medium (chemically defined serum-free medium) of human melanoma cell line UCLA-SO-M14 (M14). The isolation procedures included concentration, ultrafiltration, gel filtration chromatography, and chloroform:methanol (C:M) extraction. The melanoma TAA activity recovered from the organic phase of the C:M extract was subsequently fractionated by gel filtration and radiolabeled with Na¹²⁵I. The radioiodinated antigen was further purified by Sephacryl S-200 gel filtration and allogeneic antibody affinity chromatography. With the use of previously characterized anti-TAA allogeneic sera from melanoma patients and ¹²⁵I-labeled TAA, a radioimmunoassay (RIA) was developed. Protein A-bearing *Staphylococcus aureus* was used to separate bound and unbound ¹²⁵I-labeled TAA. The coefficient of variation between experiments and within experiments with unlabeled melanoma TAA as the competitor in the competitive RIA ranged from 8.9 to 20.4%. These variations were consistently lower (8.9-13.6%) at high levels (6 micrograms melanoma TAA/ml) of the competitor than they were (17.3-20.5%) at low levels (0.5 microgram melanoma TAA/ml), suggesting reasonable reproducibility of the assay. A logit versus log plot of the competitive RIA data and analysis by linear regression yielded a straight line. This line represented a 5- to 1,000-ng detection range for melanoma TAA. Analysis of C:M-extracted and Sephacryl S-200-purified melanoma TAA by the competitive RIA revealed a 695-fold purification of the antigen that represented a 37.5% recovery from the spent culture medium. The greatest enrichment of the melanoma TAA was achieved by the C:M extraction step. This step separated the melanoma TAA from other antigens, e.g., fetal antigen and human leukocyte antigens.

PMID: 6582304 [PubMed - indexed for MEDLINE]



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Identification and solubilization of iodinated cell surface human melanoma associated antigens.

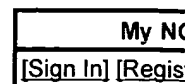
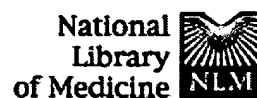
Bystryn JC, Smalley JR.

To identify soluble cell surface melanoma-associated antigens (MAA), human melanoma cells in culture were radioiodinated by the lactoperoxidase technique and solubilized in non-ionic detergent (NP-40). Labelled MAA were identified by a quantitative double-antibody antigen binding assay and unrelated labelled macromolecules by trichloroacetic acid precipitation. Detergent solubilized 95% of the macromolecule-associated radioactivity. Approximately 8%, presumably MAA, was bound specifically by anti-melanoma serum. In contrast, anti-melanoma serum bound specifically only 0.5 to 1.5% of the acid precipitable radioactivity in control cells iodinated in a similar manner. Specificity was further studied by quantitative serum absorption. Two different melanoma lines were equally effective in inhibiting specific binding of iodinated melanoma lysate, whereas 50-100 times more normal fresh lymphocytes, liver and spleen cells, cultured HeLa or colon adenocarcinoma cells, and 8 times more cultured fetal cells were required to produce similar reductions in specific binding. These studies demonstrate that cell surface human melanoma antigens that differ qualitatively and/or quantitatively from those on normal or malignant allogeneic tissues can be solubilized and identified. These antigens are shared with other melanomas, and some are also present on fetal cells.

PMID: 70412 [PubMed - indexed for MEDLINE]

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Degradation of tumor-associated antigens shed by human melanoma cells in culture.

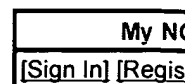
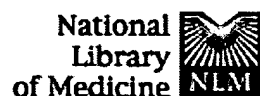
Boctor AM, Bystryn JC.

The fate of cell surface tumor-associated antigens shed by viable human melanoma cells was studied in vitro. Labeled surface material shed by radioiodinated melanoma cells was incubated with a variety of unlabeled human cells for 24 hr. Both melanoma-associated antigens (MAAs), quantitated by specific immunoprecipitation, and unrelated surface macromolecules were degraded or inactivated by normal and malignant cells including the melanoma cells themselves. The MAAs studied were particularly susceptible to degradation. Following incubation with a variety of cells, immunoreactive MAAs decreased 2 to 3 times more rapidly than did unrelated surface macromolecules shed concurrently by melanoma cells. However, melanoma cells had a selective defect in their ability to degrade MAAs. Though catabolically active, these cells degraded non-MAA surface macromolecules shed by themselves or by allogeneic cells much more rapidly than they inactivated MAAs. These observations suggest that the ultimate amount of soluble tumor antigens that accumulate in body fluids will depend on the balance between the rate of their release and that of their degradation and that as a result of a selective defect in the catabolic activity of melanoma cells some tumor antigens may be particularly prone to accumulate in the extra-cellular fluid bathing these tumors.

PMID: 7074593 [PubMed - indexed for MEDLINE]

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Monoclonal antibody defined-human melanoma-associated antigens: molecular and phylogenetic studies in normal serum.

Morgan AC Jr, McIntyre RF.

Normal human sera were analyzed for the presence and molecular form of two human melanoma-associated antigens (MAAs), the 250 "melanoma-specific" glycoprotein and the 100 K "common tumor antigen". The 250 K MAA was not synthesized by any cultures other than human melanoma and was not detectable in normal human serum. In contrast, the 100 K MAA, which is present in spent medium of cultured human melanoma, carcinoma and fetal melanocytes but not of adult normal cells, was found in normal human serum in nanogram quantities. This serum form of the 100 K MAA was also found in pooled sera of higher apes but not of lower species. The cell-derived form of the 100 K MAA, present in spent culture medium, had a similar phylogenetic distribution. The molecule was produced by cultured brain glia from gorilla, but not by melanoma cells from miniature swine or dog. The 100 K MAA from gorilla glia had a mol. wt identical to the molecule produced by human melanoma cells. Molecular characterization of this MAA in normal human serum showed that it was heterogeneous in size and was present in fractions greater than 100 kd after analytical HPLC gel sieving under non-denaturing conditions. In contrast, MAA from spent culture medium of melanoma cells was 100 kd or less in chemically defined medium (CDM) with no protein supplement, but had a higher mol. wt in CDM with BSA or fetal calf serum supplement, similar to the serum form of the molecule. An association of the 100 K MAA with albumin was demonstrated by analytical HPLC gel sieving and SDS-PAGE analysis of monoclonal antibody immunoprecipitates. The 100 K MAA was dissociated from albumin in normal human serum by treatment with SDS and fractionation by gel sieving. Under these conditions 100 K MAA from serum co-migrated with similarly treated 100 K from melanoma cells. These results indicate that the 100 K MAA is a normal serum constituent which forms a strong, non-covalent association with albumin and is evolutionarily restricted to higher apes or humans.

PMID: 6656776 [PubMed - indexed for MEDLINE]